1   1.   A method for determining the concentration of chloride ions in samples,

2 comprising:

3   preparing an enzyme reagent, said enzyme reagent including:

4   α-amylase that is substantially calcium-free; and

5   an α-amylase activity detecting substrate; and

6   combining the enzyme reagent with sodium ion and a sample containing

7 chloride ion to be assayed, the sodium ion being present in a higher concentration

8 than said chloride ion;

9   assaying the quantity of α-amylase formed due to the presence of sodium ions

10 and chloride ions in said sample; and

11   determining the quantity of said chloride ions by reference to said assay of α-

12 amylase.

13

14   2.   The method according to claim 1, wherein calcium is removed from the α-

15 amylase that is substantially calcium-free by use of a chelating compound.

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17   3.   The method according to claim 1, wherein calcium is removed from the α-

18 amylase that is substantially calcium-free by use of a compound that forms a covalent bond

19 with calcium.

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1   4.   The method according to claim 2, wherein said chelating compound is a

2 member selected from the group consisting of ethylenediaminetetraacetic acid, trans-1,2-

3 cyclohexanediamine-N,N,N′,N′-tetraacetic acid, glycol ether diamine tetraacetic acid,

4 iminotetraacetic acid, and diaminopropanetetraacetic acid.

5

6   5.   The method of claim 2, wherein said chelating compound is

7 ethylenediaminetetraacetic acid.

8

9   6.   The method according to claim 1, wherein said α-amylase activity detecting

10 substrate is a member selected from the group consisting of 2-chloro-4-nitrophenyl-α-D-

11 maltotrioside,   4-nitrophenyl-α-D-maltopentaoside   and   α-glucosidase,   2-chloro-4-

12 nitrophenyl-β-D-maltopentaoside and α-glucosidase and β-glucosidase, 4-nitrophenyl-α-D-

13 maltoheptaoside, α-glucosidase, and 2-chloro-4-nitrophenyl-β-D-maltoheptaoside and α-

14 glucosidase and β-glucosidase.

15

16   7.   The method according to claim 6, wherein said α-amylase activity detecting

17 substrate is 2-chloro-4-nitrophenyl-α-D-maltotrioside.

18

19   8.   The method according to claim 1, wherein said sample is a bodily fluid

20 sample.

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22   9.   The method according to claim 8, wherein said bodily fluid sample is

23 selected from the group consisting of serum, plasma, or urine.

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1   10.   The method of claim 1, wherein said sodium ion compound is sodium citrate.

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3   11.   The method of claim 1, wherein said sodium ion compound is sodium

4 acetate.

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1   12.   A composition for use in determining the concentration of chloride ions in a

2   fluid sample, comprising: α-amylase that is substantially calcium-free, sodium ion, and an α-

3   amylase activity detecting substrate.

4

5   13.   A composition as in claim 12 further comprising a compound capable of

6   forming a chelate with a calcium ion and a calcium chelate compound.

7

8   14.   A composition according to claim 13, wherein said compound capable of

9   forming a chelate with a calcium ion is a member selected from the group consisting of

10   ethylenediaminetetraacetic acid, trans-1,2-cyclohexanediamine-N,N,N′,N′-tetraacetic acid,

11   glycol ether diamine tetraacetic acid, iminotetraacetic acid, and diaminopropanetetraacetic

12   acid.

13

14   15.   A composition according to claim 13, wherein said compound capable of

15   forming a chelate with a calcium ion is ethylenediaminetetraacetic acid.

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17   16.   The composition according to claim 13, wherein said calcium chelate

18   compound is calcium-ethylenediaminetetraacetic acid.

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1   17.   The composition according to claim 12, wherein said α-amylase activity

2 detecting substrate is a member selected from the group consisting of 2-chloro-4-

3 nitrophenyl-α-D-maltotrioside, 4-nitrophenyl-α-D-maltopentaoside and α-glucosidase, 2-

4 chloro-4-nitrophenyl-β-D-maltopentaoside and α-glucosidase and β-glucosidase, 4-

5 nitrophenyl-α-D-maltoheptaoside, α-glucosidase, and 2-chloro-4-nitrophenyl-β-D-

6 maltoheptaoside and α-glucosidase and β-glucosidase.

7

8   18.   The composition according to claim 12, wherein said α-amylase activity

9 detecting substrate is 2-chloro-4-nitrophenyl-α-D-maltotrioside.

10

11   19.   The composition of claim 12, wherein said sodium ion compound is sodium

12 citrate.

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14   20.   The composition of claim 12, wherein said sodium ion compound is sodium

15 acetate.

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1 21. A method of activating calcium-free α-amylase for enzymatic activity

2 comprising mixing chloride ion with calcium-free α-amylase in the presence of excess

3 sodium ion.

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1   22.   A method for determining the concentration of sodium ions in samples,

2 comprising:

3   preparing an enzyme reagent, said enzyme reagent including:

4   α-amylase that is substantially calcium-free; and

5   an α-amylase activity detecting substrate; and

6   combining the enzyme reagent with excess chloride ion, and a sample

7 containing sodium ion to be assayed, the chloride ion being present in a higher

8 concentration than said sodium ion;

9   assaying the quantity of α-amylase formed due to the presence of sodium ions

10 and chloride ions in said sample; and

11   determining the quantity of said sodium ions by reference to said assay of α-

12 amylase.

13

14   23.   The method of claim 22, wherein a calcium-binding compound is combined

15 with the enzyme reagent, the excess chloride ion, and the sample containing sodium ion to

16 be assayed before the α-amylase quantity is determined.

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18   24.   The method of claim 22, wherein said calcium-binding compound is

19 ethylenediaminetetraacetic acid.

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